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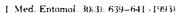
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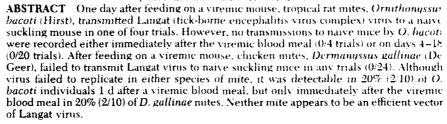
SHORT COMMUNICATION

Inefficient Mechanical Transmission of Langat (Tick-Borne Encephalitis Virus Complex) Virus by Blood-Feeding Mites (Acari) to Laboratory Mice

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LANGAT (LGT) VIRUS is a member of the tickborne encephalitis (TBE) virus complex (genus Flavivirus, family Flaviviridae). Infections in healthy persons may cause fever, but in leukemic patients this virus can produce encephalitis (Webb et al. 1966, Begum 1969). LGT virus has been isolated from three species of ixodid ticks: Haemaphysalis papuana Thorell in Thailand, Ixodes granulatus Supino in Malaysia, and Ixodes persulcatus Schulze in central Siberia (Smith 1956, Bancroft et al. 1976, Karabatsos 1985). This virus has also been isolated from Malaysian forest rats, which may serve as reservoir hosts (Smith 1956, Karabatsos 1985). Transmission of LGT virus by tick bite has been demonstrated experimentally for Ixodes ricinus (L.) and Haemaphysalis spinigera Neumann, and replication and transstadial transmission of the virus has been demonstrated in Dermacentor marginatus (Sulzer) (Varma & Smith 1962, Karabatsos 1985).

genically similar TBE (tick-borne encephalitis) virus has been isolated from hematophagous mesostigmatid mites in nature and has also been shown to persist in experimentally infected mites for up to 63 d (Naumov & Gutova 1984, Kocianova & Kozuch 1988). Therefore, the potential for two species of widely distributed hematophagous mesostigmatid mites to transmit LGT virus from viremic to naive suckling mice was investigated under laboratory conditions.

Separate, virus-free laboratory colonies of the tropical rat mite, Ornithonyssus bacoti (Hirst),

No isolations of LGT virus have been reported from blood-feeding mites. However, the anti-

Separate, virus-free laboratory colonies of the tropical rat mite, Ornithonyssus bacoti (Hirst), and the chicken mite, Dermanyssus gallinae (De Geer), were maintained as described previously (Durden & Linthicum 1992, Durden et al. 1992). One-day-old suckling mouse (Harlan-Sprague ICR strain) littermates were inoculated intraperitoneally with 103.3 plaque-forming units (PFU) of the TP 21 strain of LGT virus. This strain, obtained from the Yale Arbovirus Research Unit, was originally isolated from I. granulatus collected in the Ulu Langat Forest reserve, Malaysia, in 1956'(Smith 1956) and had been passaged eight times in suckling mice and three times in Vero cells before use in these experiments. Three days after inoculation, eight of the LGT-inoculated mice were removed and each was placed into a separate mite-proof container. Sixty unfed, uninfected, female O. bacoti were allowed to feed on each of four of these

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care. The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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mice, while 60 unfed, uninfected, female D. gallinge were allowed to feed on each of the remaining four mice. Mites were allowed to feed for 2 h, at which time each mouse was anesthetized with CO₂ and blood (0.1 ml) was collected by cardiac puncture. Blood was mixed 1:10 in diluent (10% fetal bovine serum in Medium 199 with Hanks' salts and antibiotics) and frozen at -70°C. Immediately after mites had fed on the viremic mouse and on days 1, 4, 8, 12, and 18, 8-10 of the virus-exposed mites of each species were allowed to feed for 2 h on a naive suckling mouse (one mouse per trial for each species of mite). These suckling mice were then returned to the dam and monitored daily over the next 21 d for signs of viral infection. Moribund mice were killed with CO2, blood and brain samples were collected, and tissue suspensions were prepared by mixing 1:10 in diluent and then frozen at -70°C. After 21 d. surviving test mice were anesthetized with CO₂ and 0.5-1 ml of blood was collected by cardiac puncture. These blood samples were allowed to clot at room temperature and sera were removed and tested for specific IgG antibody to LGT virus by using an enzyme-linked immunosorbent assay (ELISA). Immediately after they had fed on a viremic mouse or on days 1, 4, 8, 12, and 18, individual mites were triturated in 1 ml of diluent and frozen at -70°C. Blood, brain, and mite samples were thawed at a later date, clarified by centrifugation at $1,000 \times g$ for 10 min, and tested for virus by plaque assay (Earley et al. 1967) on confluent monolayers of 4- to 5-d-old LLC-MK, cells (a rhesus monkey kidney cell line). Blood and brain samples of moribund mice were also tested by ELISA for LGT virus.

Viremia levels in the eight virus-inoculated suckling mice immediately after mite feeding were 107.1 to 107.5 PFU per ml of blood. Virus was transmitted by O. bacoti to a naive suckling mouse in one of four trials I d after the mites had fed on a viremic suckling mouse. No other transmissions were detected (0/20 for O. bacoti, 0/24 for D. gallinae). The single transmission by o bacoti was evidenced by a moribund suck-Juse 4 d after virus-exposed mites had fed lir and by positive plaque-assay (10^{7.9} PFU per ml of brain tissue) and ELISA results. One additional suckling mouse that was fed upon by virus-exposed O. bacoti became moribund, but brain and blood samples were negative for virus (cause of morbidity unknown). All 21-d sera from surviving mice were negative for specific IgG antibody to LGT virus by ELISA.

Virus was detected in 50% (5/10) (mean positive titer = 101.6 PFU per mite) of the O. bacoti sampled immediately after they had fed on a viremic mouse. This percentage dropped to 20% (2/10, mean positive titer = 10^{1.2} PFU per mite) after 1 d, and to 0% (0/40) after 4-18 d. Comparable data for D. gallinae were 20% (2/10, mean positive titer = $10^{1.2}$ PFU per mite) immediately after the viremic blood meal and 0% (0/50) after

Neither O. bacoti nor D. gallinae was an efficient vector of LGT virus in our transmission trials. The failure of LGT virus to replicate in either species of mite indicates that the single transmission by O. bacoti 1 d after the viremic meal was probably mechanical rather than biological. These data suggest that blood-feeding mites are probably not involved in the natural transmission cycle of LGT virus.

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